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(54) Title: CCK ANALOGS CONTAINING α-SUBSTITUTED AMINO ACIDS

(57) Abstract

The invention covers novel peptides which contain at least one α,α-disubstituted amino acid. The compounds are useful as agents in the treatment of obesity and gastrointestinal disorders associated with gastrin. They are also useful in treating gastrin-dependent tumors, or as antipsychotics. Further, the compounds are antianxiety agents, antiulcer agents, antidepressant agents, and are agents useful for preventing the withdrawal response produced by chronic treatment or use followed by chronic treatment followed by withdrawal from nicotine, diazepam, alcohol, cocaine, caffeine, or opioids. Also covered are processes for making the compounds, novel intermediates useful in their preparation, compositions containing them, and methods of using the compounds.

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CCK ANALOGS CONTAINING α-SUBSTITUTED AMINO ACIDS

BACKGROUND OF THE INVENTION

Agents acting at central cholecystokinin (CCK) receptors may induce satiety (Schick, Yaksh and Go, Regulatory Peptides 14:277-291 (1986).

The CCK peptides are widely distributed in various organs of the body including the gastrointestinal tract, endocrine glands, and the nerves of the peripheral and central nervous systems. Various biologically active forms have been identified including a 33-amino acid hormone and various carboxyl-terminus fragments of this peptide (e.g., the octapeptide CCK26-33 and the tetrapeptide (CCK30-33). (G. J. Dockray, Br. Med. Bull., 38 (No. 3):253-258, 1982).

The various CCK peptides are thought to be involved in the control of smooth muscle contractility, exocrine and endocrine gland secretion, sensory nerve transmission, and numerous brain functions. Administration of the native peptides cause gall bladder contraction, amylase secretion, excitation of central neurons, inhibition of feeding, anticonvulsive actions, and other behavioral effects. ("Cholecystokinin: Isolation, Structure and Functions," G. B. J. Glass, Ed., Raven Press, New York, 1980, pp 169-221; J. E. Morley, Life Sciences 27:355-368, 1980; "Cholecystokinin in the Nervous System," J. de Belleroche and G. J. Dockray, Ed., Ellis Horwood, Chichester, England, 1984, pp 110-127.)

The high concentrations of CCK peptides in many brain areas also indicate major brain functions for these peptides (G. J. Dockray, Br. Med. Bull., 38

(No. 3):253-258, 1982). The most abundant form of brain CCK found is CCK26-33, although small quantities of CCK30-33 exist (Rehfeld and Gotterman, J. Neurochem. 32:1339-1341 (1979)). The role of central nervous system CCK is not known with certainty, but it has been implicated in the control of feeding (Della-Fera and Baile, Science 206:471-473 (1979)).

Currently available appetite suppressant drugs either act peripherally, by increasing energy expenditure (such as thyroxine), or in some other manner (such as the biguanides), or act by exerting a central effect on appetite or satiety.

Recently patients with bulemia were shown to have lower than normal CCK levels in their plasma (Geracioti, et al, New England Journal of Medicine 319:683 (1988)). An additional role for CCK in the periphery is to regulate the release of insulin. CCK has been shown to increase the levels of insulin when administered to mammals (Rushakoff, et al, J. Clin. Endocrinol. Metab. 65:395 (1987)).

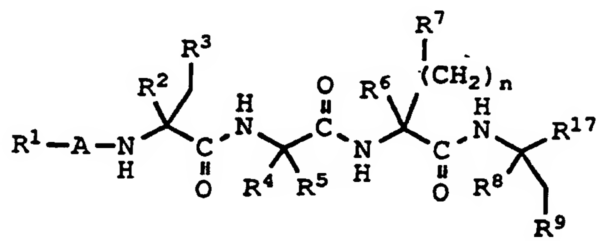
C-terminal fragments of CCK have recently been reported to function as CCK receptor antagonists (Jensen, et al, Biochem. Biophys. Acta 757:250 (1983); Spanarkel, J. Biol. Chem. 258:6746 (1983)). Japanese patent application 70/10506 to Miyao, et al, discloses a tetrapeptide derivative of the carboxy terminal sequence of gastrin (L-Trp-L-Lys-L-Asp-L-Phe-NH_2) which has antigastrin activity.

Centrally acting appetite suppressants either potentiate central catecholamine pathways and tend to be stimulants (for example, amphetamine), or influence serotonergic pathways (for example, fenfluramine). Other forms of drug therapy include bulking agents which act by filling the stomach, thereby inducing a "feeling" of satiety.

WO 90/06937 covers derivatives of tetrapeptides as CCK agonists. The compounds are for treating gastrointestinal disorders, central nervous system disorders, insulin-related disorders, treatment of pain or regulating appetite.

SUMMARY OF THE INVENTION

The invention relates to novel compounds which are CCK ligands and are α,α-substituted mono-, di-, tri-, tetra-, or polypeptides of formula



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and the pharmaceutically acceptable salts thereof wherein R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{17}, A, and n are as defined below.

In commonly assigned copending application 07/690,755 filed April 24, 1991, and its continuation-in-part application 07/852,086 filed March 20, 1992, by Horwell, et al, the disclosure of which is incorporated by reference, α-substituted polypeptides are disclosed.

The invention also relates to a pharmaceutical composition containing an effective amount of a compound according to formula I in combination with a pharmaceutically acceptable carrier in unit dosage form effective for appetite suppression.

The invention further relates to a method of appetite suppression in mammals which comprises administering an amount effective to suppress appetite of the composition described above to a mammal in need of such treatment.

The invention further relates to methods of treating gastrointestinal disorders, central nervous system disorders such as CNS suppressants which can exhibit such effects as antipsychotic, neuroleptic, anxiolytic, and anticonvulsant.

The invention further relates to methods of reducing gastric acid secretion and to treating gastrointestinal ulcers.

The invention further relates to blocking the reaction caused by withdrawal from drug or alcohol use and to potentiating the effects of morphine and other opioids in treating pain.

The invention further provides processes for the preparation of compounds of formula I.

The invention further provides novel intermediates useful in the preparation of compounds of formula I and also provides processes for the preparation of the intermediates.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows Compound 2 is a partial agonist.

Figure 2 shows Compound 4 is an agonist and Compound 5 is a weak agonist.

Figure 3 shows Compound 6 is an agonist.

Figures 1 through 4 show CCK8s as agonist reference standards.

DETAILED DESCRIPTION

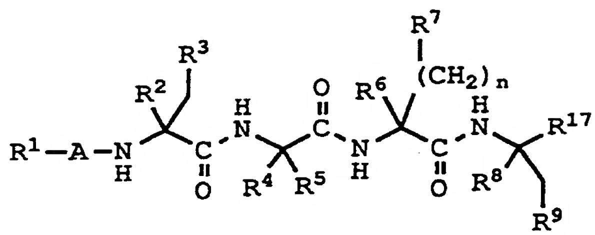
The following table provides a dictionary of the terms used in the description of the invention.

TABLE I

Abbreviated Designation	
GLY	Glycine
ß-ALA	ß-Alanine
GABA	4-Aminobutanoic Acid
DAVA	δ-Aminovaleric Acid
MET	L-Methionine
TYR	L-Tyrosine
ASP	L-Aspartic Acid
PHE	L-Phenylalanine
TRP	L-Tryptophan
BOC	Tertbutoxycarbonyl
1-ADOC	1-Adamantyloxycarbonyl
2-ADOC	2-Adamantyloxycarbonyl
Z	Benzyloxycarbonyl
FMOC	9-Fluorenylmethoxycarbonyl
Aib	α-Aminoisobutyric Acid
PFP	Pentafluorophenyl

The compounds of the present invention are analogs of CCK 30-33 and CCK 26-33 which contain at least one residue of an amino acid which is α,α-disubstituted. These compounds differ from the mammalian genetically coded natural peptides in that the α-substituents cannot all simultaneously be hydrogen.

The compounds of the present invention are represented by the formula



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or a pharmaceutically acceptable salt thereof wherein

R^1 is hydrogen, BOC, 1-Adoc, 2-Adoc, Z, FMOC, $R^{12}R^{13}R^{14}Si(CH_2)_mOCO$- wherein R^{12}, R^{13}, and R^{14} are each independently lower alkyl of from 1 to 3 carbon atoms and m is an integer of from 0 to 3, $R^{15}CO$- or $R^{15}NHCO$- wherein R^{15} is a straight or branched alkyl of from 1 to 6 carbons or 1- or 2-adamantyl;

A is a bond, Gly, ß-ALA, GABA, DAVA, Aib, $NH(CH_2)_p$ wherein p is an integer of from 1 to 6,
NHCO,
Met-Gly,
Tyr-Met-Gly,
Asp-Tyr(OSO_3H),
Asp-Tyr-Met-Gly,
Asp-Tyr(OSO_3H)-Met-Gly;

R^2, R^4, R^6, and R^8 are each independently hydrogen with the proviso that not all are simultaneously hydrogen, lower alkyl, $-CH=CH_2$, $C\equiv CH$, $-CH_2-CH=CH_2$, $-CH_2C\equiv CH$, $-CH_2Ar$, $-CH_2OR$, $-CH_2OAr$, $-(CH_2)_qCO_2R$, $-(CH_2)_qN(R)_2$ wherein q is an integer of from 0 to 3, R is hydrogen or lower alkyl, Ar is a mono- or polycyclic unsubstituted or substituted carbo- or heteroaromatic or hydroaromatic moiety;

R^3 is 2- or 3-indolyl, 1- or 2-naphthyl, 2- or 3-benzothienyl, 2- or 3-benzofuranyl, 2- or 3-indazoyl or phenyl, any of these can have from 0 to 3 substituents each independently selected from hydrogen, fluorine, chlorine, bromine, iodine, methyl, methoxy, trifluoromethyl, nitro, hydroxy, NH_2, and OCF_3;

R^5 is lower alkyl, $(CH_2)_rS(CH_2)_sCH_3$ wherein r is an integer of from 1 to 6 and s is an integer of from 0 to 6;

n is an integer of from 1 to 3;

R^7 is -OH,

-COOH,

tetrazole,

triazole,

$-COOR^{16}$,

$-CONR^{10}R^{11}$,

wherein R^{16} is lower alkyl, and R^{10} and R^{11} are as described below;

R^9 is selected from R^3 above;

R^{17} is $-\overset{O}{\overset{\|}{C}}-NR^{10}R^{11}$, CH_2OR^{18} or H wherein

R^{10} and R^{11} are each independently hydrogen, lower alkyl, alkoxycarbonyl, carboxyalkyl, or R^{10} and R^{11} together form a ring of from 3 to 10 atoms with the nitrogen to which they are attached which ring contains atoms selected from carbon, nitrogen, oxygen, and sulfur; and

R^{18} is R^1 or CH_2CO_2H or $CH_2CONR^{10}R^{11}$ or $CONR^{10}R^{11}$;

R^2 and R^3, R^4 and R^5, R^6 and R^7, R^8 and R^9 or R^9 and R^{10} may together form a ring of from 3 to 8 atoms which atoms are selected from carbon, nitrogen, oxygen, and sulfur.

Preferred compounds of the instant invention are those of formula I wherein

R^1 is Fmoc, Z, Boc, or $Me_3Si(CH_2)_2OCO$-;

A is a bond, Gly, ß-ALA, Aib, Asp-Tyr-Met-Gly, Asp-Tyr(OSO_3H)-Met-Gly;

R^2, R^4, R^6, and R^8 are each independently alkyl of from 1 to 3 carbon atoms, carboxyalkyl of 1 or 2 carbon atoms, or CH_2OH;

R^3 is 3-indazolyl, 3-benzothienyl, 2-benzofuranyl, 3-benzofuranyl, or 2-bromo-3-benzofuranyl;

R^5 is isobutyl, sec-butylethyl, n-propyl, or i-propyl;

R^7 is $CONH_2$, $CONMe_2$, or OH;

R^9 is selected from R^3;

R^{10} and R^{11} are each independently alkyl of from 1 to 3 carbon atoms or together R^{10} and R^{11} form a ring of from 4 to 8 atoms including the nitrogen to which they are attached.

More preferred compounds of the instant invention are those of formula wherein

R^1 is hydrogen or BOC;

A is a bond or ß-ALA;

R^2, R^4, R^6, and R^8 are each independently CH_3 or CH_2CO_2H;

R^3 is 3-indolyl, 1-naphthyl, or 2-naphthyl;

R^5 is n-butyl, n-pentyl, $(CH_2)_2SCH_3$ or $(CH_2)_2SCH_2CH_3$;

R^7 is OH, tetrazole, triazole, or CO_2H;

R^9 is phenyl, 1-naphthyl, or 2-naphthyl;

R^{10} and R^{11} are each independently hydrogen, CH_3 or together R^{10} and R^{11} form a ring of 5 or 6 atoms including the nitrogen to which they are attached.

Especially preferred compounds of the instant invention are selected from the group consisting of:

α-Methyl-DL-tryptophyl-L-methionyl-L-α-aspartyl-L-phenylalaninamide,

α-Methyl-D-tryptophyl-L-methionyl-L-α-aspartyl-L-phenylalaninamide,

α-Methyl-L-tryptophyl-L-methionyl-L-α-aspartyl-L-phenylalaninamide,

N-[[(1,1-dimethylethoxy)-carbonyl]-α-methyl]-L-tryptophyl]-L-methionyl-L-α-aspartyl-L-phenylalaninamide,

N-[(1,1-dimethylethoxy)-carbonyl]-α-methyl-DL-tryptophyl-L-methionyl-L-α-aspartyl-L-phenylalaninamide,

L-tryptophyl-2-methyl-DL-methionyl-L-α-aspartyl-L-phenylalaninamide,

N-[(1,1-dimethylethoxy)-carbonyl]-α-methyl-D-tryptophyl-L-methionyl-L-α-aspartyl-L-phenylalaninamide,

Glycyl-α-methyl-D-tryptophyl-L-methionyl-L-α-aspartyl-L-phenylalaninamide,

Glycyl-α-methyl-DL-tryptophyl-L-methionyl-L-α-aspartyl-L-phenylalaninamide,

Glycyl-α-methyl-L-tryptophyl-L-methionyl-L-α-aspartyl-L-phenylalaninamide,

Trp-Met-Asp-DL-MePhe-$NH_2 \cdot CF_3CO_2H$, and

Trp-Met-DL-MeAsp-Phe-$NH_2 \cdot CF_3CO_2H$.

The D and the L configuration of the compounds of formula I are possible at the chiral centers and are included in the scope of the invention.

Preferred compounds are those wherein the α-amino acid residues are of the [L] configuration.

The compounds of the present invention can have multiple chiral centers depending on their structures. Centers of asymmetry may exist on carbon atoms bearing

substituents R^1 in formula I and at R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, and R^{17} in formula I. In particular, the compounds of the present invention may exist as diastereomers, mixtures of diastereomers, or as the mixed or the individual optical enantiomers. The present invention contemplates all such forms of the compounds. The mixtures of diastereomers are typically obtained as a result of the reactions described more fully below. Individual diastereomers may be separated from mixtures of the diastereomers by conventional techniques such as column chromatography or repetitive recrystallizations.

The compounds of the instant invention include the solvates and hydrates and pharmaceutically acceptable salts of formula I.

The term lower alkyl means straight or branched chain alkyl groups of from 1 to 6 carbon atoms unless otherwise specified.

The term alkoxycarbonyl means a carbon group of from 1 to 4 carbon atoms.

Preferred pharmaceutically acceptable salts are benzathine, chloroprocaine, choline, diethanolamine, ethylenediamine, meglumine, procaine, aluminum, calcium, lithium, magnesium, potassium, sodium, zinc, diethylamine, and tromethane.

Especially preferred pharmaceutically acceptable salts are N-methylglucamine and sodium for acids and HCl, sulfate, and trifluoroacetate for bases.

Individual enantiomers may be separated by conventional methods well known in the art such as conversion to a salt with an optically active compound, followed by separation by chromatography or recrystallization and reconversion to the nonsalt form.

The preferred stereochemistry of the compounds of the invention is that exhibited by the compound of Example 2.

The compounds of the present invention can be formed by coupling individual substituted α-amino acids by methods well known in the art. (See, for example, standard synthetic methods discussed in the multi-volume treatise "The Peptides, Analysis, Synthesis, Biology," by Gross and Meinhofer, Academic Press, New York.) The individual substituted alpha amino acid starting materials are generally known or, if not known, may be synthesized and, if desired, resolved by methods within the skill of the art. (Synthesis of racemic [DL]-α-methyl tryptophan methyl ester - see Braña, M. F., et al, J. Heterocyclic Chem. 17:829 (1980)).

The invention also includes novel intermediates which are useful in the preparation of the final products. These intermediates are the following compounds or the compound on the solid phase resin; for example, Fmoc-MePhe-Resin.

Fmoc-MePhe-NH_2,
Fmoc-Asp(OBu^t)-MePhe-NH_2,
Fmoc-Met-Asp(OBu^t)-MePhe-NH_2,
Fmoc-Trp-Met-Asp(OBu^t)-MePhe-NH_2,
Asp(OBu^t)-MePhe-NH_2,
Asp-MePhe-NH_2,
Fmoc-Met-Asp-MePhe-NH_2,
Met-Asp(OBu^t)-MePhe-NH_2,
Met-Asp-MePhe-NH_2,
Trp-Met-Asp(OBu^t)-MePhe-NH_2,
Fmoc-Trp-Met-Asp-MePhe-NH_2,
Fmoc-MeAsp(OBu^t)-Phe-NH_2,
Fmoc-MeAsp-Phe-NH_2,
Fmoc-Met-MeAsp(OBu^t)-Phe-NH_2,

Fmoc-Trp-Met-MeAsp(OBu^t)-Phe-NH_2,
MeAsp(OBu^t)-Phe-NH_2,
MeAsp-Phe-NH_2,
Met-MeAsp(OBu^t)-Phe-NH_2,
Met-MeAsp-Phe-NH_2,
Fmoc-Met-MeAsp-Phe-NH_2,
Fmoc-Trp-Met-MeAsp-Phe-NH_2,
MeMet-Asp(OBu^t)-Phe-NH_2,
Fmoc-MeMet-Asp(OBu^t)-Phe-NH_2,
Fmoc-MeMet-Asp-Phe-NH_2
MeMet-Asp-Phe-NH_2,
Fmoc-Trp-MeMet-Asp(OBu^t)-Phe-NH_2,
Fmoc-Trp-MeMet-Asp-Phe-NH_2,
Fmoc-MeTrp-Met-Asp(OBu^t)-Phe-NH_2,
Fmoc-MeTrp-Met-Asp-Phe-NH_2,
Fmoc-Gly-Trp-Met-Asp-MePhe-NH_2,
Fmoc-Gly-Trp-Met-Asp(OBu^t)MePhe-NH_2,
Gly-Trp-Met-Asp(OBu^t)MePhe-NH_2,
Fmoc-Gly-Trp-Met-MeAsp-Phe-NH_2,
Fmoc-Gly-Trp-Met-MeAsp(OBu^t)-Phe-NH_2,
Gly-Trp-Met-MeAsp(OBu^t)-Phe-NH_2,
Fmoc-Gly-Trp-MeMet-Asp-Phe-NH_2,
Fmoc-Gly-Trp-MeMet-Asp(OBu^t)-Phe-NH_2,
Gly-Trp-MeMet-Asp(OBu^t)-Phe-NH_2,
Fmoc-Gly-MeTrp-Met-Asp-Phe-NH_2,
Fmoc-Gly-MeTrp-Met-Asp(OBu^t)-Phe-NH_2,
Gly-MeTrp-Met-Asp(OBu^t)-Phe-NH_2,
Fmoc-MeMet-OH,
Fmoc-MeMet-OPFP,
MeAsp(OBu^t)OH,
Fmoc-MeAsp(OBu^t)OH, and
Fmoc-MeAsp(OBu^t)OPFP.

For preparing pharmaceutical compositions from the compounds of this invention, inert, pharmaceutically acceptable carriers can be either

solid or liquid. Solid form preparations include powders, tablets, dispersible granules, capsules, cachets, and suppositories.

A solid carrier can be one or more substances which may also act as diluents, flavoring agents, solubilizers, lubricants, suspending agents, binders, or tablet disintegrating agents; it can also be an encapsulating material. In powders, the carrier is a finely divided solid which is in a mixture with the finely divided active component. In tablets, the active component is mixed with the carrier having the necessary binding properties in suitable proportions and compacted in the shape and size desired.

For preparing suppository preparations, a low-melting wax such as a mixture of fatty acid glycerides and cocoa butter is first melted and the active ingredient is dispersed therein by, for example, stirring. The molten homogeneous mixture is then poured into convenient sized molds and allowed to cool and solidify.

The powders and tablets preferably contain 5 to about 70% of the active component. Suitable carriers are magnesium carbonate, magnesium stearate, talc, lactose, sugar, pectin, dextrin, starch, tragacanth, methyl cellulose, sodium carboxymethyl cellulose, a low-melting wax, cocoa butter, and the like.

The term "preparation" is intended to include the formulation of the active component with encapsulating material as a carrier providing a capsule in which the active component (with or without other carriers) is surrounded by a carrier which is thus in association with it. Similarly, cachets are included.

Tablets, powders, cachets, and capsules can be used as solid dosage forms suitable for oral administration.

Liquid form preparations include solutions, suspensions, and emulsions. Sterile water or water-propylene glycol solutions of the active compounds may be mentioned as an example of liquid preparations suitable for parenteral administration. Liquid preparations can also be formulated in solution in aqueous polyethylene glycol solution.

Aqueous solutions for oral administration can be prepared by dissolving the active component in water and adding suitable colorants, flavoring agents, stabilizers, and thickening agents as desired. Aqueous suspensions for oral use can be made by dispersing the finely divided active component in water together with a viscous material such as natural synthetic gums, resins, methyl cellulose, sodium carboxymethyl cellulose, and other suspending agents known to the pharmaceutical formulation art.

Preferably, the pharmaceutical preparation is in unit dosage form. In such form, the preparation is divided into unit doses containing appropriate quantities of the active component. The unit dosage form can be a packaged preparation, the package containing discrete quantities of the preparation, for example, packeted tablets, capsules, and powders in vials or ampoules. The unit dosage form can also be a capsule, cachet, or tablet itself, or it can be the appropriate number of any of these packaged forms.

The biological activity of compounds of the present invention was evaluated employing an initial screening test which rapidly and accurately measured the binding of the tested compound to be known CCK receptor sites. Specific CCK receptors have been shown to exist in the central nervous system. (See Hays et al, Neuropeptides 1:53-62 (1980); and Satuer et al, Science 208:11555-56 (1980).

In this screening test, the cerebral cortices taken from male CFLP mice weighing between 30 to 40 g were dissected on ice, weighed, and homogenized in 10 volumes of 50 mM Tris-HCl buffer (pH 7.4 at 0-4°C). The resulting suspension was centrifuged, the supernate was discarded, and the pellet was washed by resuspension in Tris-HCl buffer followed by recentrifugation. The final pellet was resuspended in 20 volumes of 10 nM Hepes buffer (pH 7.2 at 23°C) containing 130 nM NaCl, 4.7 nM KCl, 5 nM $MgCl_2$, 1 nM EDTA, 5 mg/mL bovine albumin, and bacitracin (0.25 mg/mL).

In saturation studies, cerebral cortical membranes were incubated at 23°C for 120 minutes in a final volume of 500 μliter of Hepes incubation buffer (pH 7.2) together with 0.2-20 nM tritiated-pentagastrin (Amersham International, England).

In the displacement experiments, membranes were incubated with a single concentration (2 nM) of ligand, together with increasing concentrations (10^{-11} to 10^{-14}M) of competitive test compound. In each case, the nonspecific binding was defined as that persisting in the presence of the unlabeled octapeptide CCK_{26-33} (10^{-6}M).

Following incubation, radioactivity bound to membranes was separated from that free in solution by rapid filtration through Whatman GF/B filters and washed three times with 4 mL of ice cold Tris-HCl buffer. Filters from samples incubated with tritiated-pentagastrin were placed in polyethylene vials with 4 mL of scintillation cocktail, and the radioactivity was estimated by liquid scintillation spectrometry (efficiency 47-52%).

The specific binding to CCK receptor sites was defined as the total bound tritiated-pentagastrin

minus the amount of tritiated-pentagastrin bound in the presence of 10^{-6} octapeptide, CCK_{26-33}.

Saturation curves for specific tritiated-pentagastrin binding to mouse cortical membranes were analyzed by the methods of Scatchard (Ann. New York Acad. Sci. 51:660-72 (1949), and Hill (J. Physiol. 40:IV-VIII (1910), to provide estimates for the maximum number of binding sites (B_{max}) and the equilibrium dissociation constant (K_a).

In displacement experiments, inhibition curves were analyzed by either logit-lot plots or the iterative curve fitting computer program ALLFIT (DeLean, Munson and Redbard, 1978) to provide estimates of the IC_{50} and nH (apparent Hill coefficient) values). IC_{50} values were defined as the concentration of test compound required to produce 50% inhibition of specific binding.)

The inhibition constant (K_i) of the test compounds was then calculated according to the Cheng-Prusoff equation:

$$K_i = \frac{IC_{50}}{1 + [L]/K_a}$$

wherein [L] is the concentration of radiolabel and K_a is the equilibrium dissociation constant.

The K_i/M values for several representative compounds of the present invention are present in Table II.

TABLE II. Binding to Central CCK Receptors

Compound	K_i/nM	(n)
(1)	7.16 ± 1.95	9
(2)	5.44 (4.23-7.10)	3
(3)		
(4)	7.80 (5.56-12.9)	3
(5)	9.20 (6.86-11.8)	3
(6)	30.40 (26.6-35.5)	3
(7)		

(n) = Number of assays

Electrophysiological Data

Explanations and protocols for similar experiments to these can be found in J. Hughes, Proc. Natl. Acad. Sci. 87:6728-6732 (1990), and references cited therein.

Figure 1 shows the dose response curve for CCK8s before and during exposure to compound (2) and a curve for compound (2) alone. The curves represent the total number of action potentials occurring during a response to CCK8s or (2) and are plotted as counts (Y-axis) versus dose (X-axis). The curves for compound (2) indicate that this compound has partial agonist type properties in this experiment. --•-- is CCK-8s, ==■== is (2), and ....▲.... is CCK-8s + (2) (1 μM).

Figure 2 shows the dose response curves for BH-CCK and CCK4 analogs on the same neurone in the VMH. The total number of action potentials occurring during a response is plotted as counts (Y-axis) versus dose of ligand (X-axis). Curves for the function (X*A)/(X+B) were fitted to the points using RS1 software (A = maximum possible response, B = EC_{50} of compound calculated from curve). The curves indicate

that both (4) and (5) have agonist-like properties in this experiment. ==o== is (5), ...Δ.... is BH-CCK, ----▲---- is (4), - - - - is (1078.292444*X)/(5.395304e-03+X), ________ is (1149.663878X*)/(0.010686+X), ... is (1100*X)/(X+0.47953), ------- is (1100*X)/(X+0.014358), ... is (0.5) smoothed CCK, and • is CCK.

In Figures 3 and 4 the dose response curves for CCK and for (4), (5), and (6) both before and after exposure to the potent CCK-8 antagonist PD 134308. The total number of action potentials occurring during a response is plotted as counts (Y-axis) versus dose (X-axis). Curves for the function (X*A)/(X+B) were fitted to the points using RS1 software. These curves indicate that compounds (4), (5), and (6) have CCK agonist-like properties which are antagonized by CCK-8 antagonists.

In Figure 3 --•-- is CCK, ==▲== is (8), ----Δ---- is (6) + PD 134308, ------- is (X*1126.907666)/(X+9.879552), ________ is (1123*X)/(X+0.140626), and ======= is (1127*X)/(X+0.031476).

In Figure 4 --•-- is CCK, ==■== is (5), ...▲.... is (4), ----□---- is (5) + PD 134308, and ----Δ---- is (4) + PD 134308.

Tables III and IV summarize the four figures.

TABLE III

Compound	Profile	Figure	EC_{50}/nM
(2)	Partial agonist	1	--
(4)	Agonist	2	14.0
(5)	Weak agonist	2	479.5
(6)	Agonist	3	524.0
CCK8s	Agonist	1-4	5.4

The compounds were further proven to be agonists following experiments in which they were challenged with the previously described potent and selective CCK-B antagonist, reference compound PD 134308 named [R-(R*,R*)-4-[[2-[[3-(1H-indol-3-yl)-2-methyl-1-oxo-2-[[(tricyclo[3.3.1.$1^{3.7}$]dec-2-yloxy)carbonyl]amino]-propyl]amino]-1-phenethyl]amino]-4-oxobutanoic acid.

TABLE IV

Compound	Graph	K_e (PD 134308)/nM
(4) + reference compound	4	4.67
(5) + reference compound	4	3.02
(6) + reference compound	3	14.4
CCK8s + reference compound	--	5.4

Data shows that compound (4) is equipotent with CCK8s while compound (6) possesses slightly lower electrophysiological activity and compound (5) has relatively low agonist activity. Compound (2) is a partial agonist.

Compounds of the present invention are useful as appetite suppressants as based on the tests described hereinbelow.

In the Palatable Diet Feeding assay, adult male Hooded Lister rats weighing between 200-400 g are housed individually and trained to eat a palatable diet. This diet consists of Nestlés sweetened condensed milk, powdered rat food, and rat water which was when blended together set to a firm consistency. Each rat is presented with 20 to 30 g of the palatable diet for 30 minutes per day during the light phase of the light-dark cycle over a training period of 5 days. The intake of palatable diet is measured by weighing the food container before and after the 30-minute access period (limits of accuracy 0.1 g). Care is taken to collect and correct for any spillage of the diet. Rats have free access to pellet food and water except during the 30-minute test period.

After the training period, dose-response curves are constructed for CCK8 and several representative compounds of the present invention (n = 8-10 rats per dose level). MPE_{50} values (±95% confidence limits) are obtained for the anorectic effects of these compounds.

In therapeutic use as appetite suppression agents, the compounds of the instant invention are administered to the patient at dosage levels of from about 200 to about 2800 mg per day.

As agonists the compounds of the instant invention have therapeutic utility as appetite suppressants and, as antagonists, as agents in treating gastrointestinal disorders, central nervous system disorders such as CNS suppressants which can exhibit such effects as antipsychotics, neuroleptics, anxiolytics, and anticonvulsants.

As antagonists, the compounds of the instant invention are expected to have utility in reducing gastric acid secretion, in reducing anxiety, in treating gastrointestinal ulcers, in treating psychotic behavior, in blocking the reaction caused by withdrawal from drug, alcohol, cocaine, benzodiazepine, diazepam, or nicotine, and in potentiating the effects of morphine for treating pain.

CCK Analogs containing α-methyl amino acids were made by three methods. Method A is a solution phase procedure wherein the peptides were assembled using standard solution phase protocols yielding fully protected intermediates or standard salt coupling which yield aspartic acid side-chain deprotected intermediates. Examples 1 and 2 below illustrate Method A.

The peptides can also be prepared by Method B, a solid-phase method wherein peptides were constructed on solid-phase resins designed to produce C-terminal amides either by treatment of the resin with ammonia in methanol or by direct cleavage of an appropriately substituted resin using trifluoroacetic acid, with the required scavengers, giving the amides directly. The latter protocol was used with DuPont RapidAmide® or Nova Biochem Novasyn KR® resins either in a simple bubbler apparatus (DuPont resin) or automated synthesizer (Nova Biochem resin). This method is illustrated by Example 3 below.

The third method, Method C, is a nonsolvent method whereby the amino component and a pentafluorophenyl ester are heated together without a solvent at 50° to 100°C for 10 to 120 minutes or more. This method is illustrated by Example 4 below.

Male Hooded Lister rats (175-250 g) are housed individually and fasted overnight (free access to water). They are anesthetized with urethane (1.5 g/kg IP) and the trachea cannulated to aid spontaneous respiration. The stomach is perfused continuously using a modification of the original method of Ghosh & Schild in "Continuous recording of acid secretion in the rat", Br. J. Pharmac. 13:54-61, 1956 as described by Parsons in "Quantitative studies of drug-induced gastric acid secretion". (Ph.D. Thesis, University of London, 1969). The cavity of the stomach is perfused at a rate of 3 mL/min with 5.4% w/v glucose solution through both the esophageal and body cannula. The fluid is propelled by a roller pump (Gilson, Minipuls 2), through heating coils to bring its temperature to 37 ± 1°C. The perfusion fluid is collected by the fundic collecting funnel and passed to a pH electrode connected to a Jenway pH meter (PHM6). An output is taken from the pH meter to a Rikadenki chart recorder for the on-line recording of the pH of the gastric perfusate.

Pentagastrin is stored as a frozen aliquot and diluted to the required concentrations with sterile 0.9% w/v NaCl. Novel compounds are dissolved in sterile 0.9% w/v NaCl on the day of the experiment. Drugs are administered IV through a cannulated jugular vein as a bolus in a dose volume of 1 mL/kg washed in with 0.15 mL 0.9% w/v NaCl. Basal pH is allowed to stabilize before administration of compounds is begun. Typically 30 minutes elapses between surgery and the first compound administration.

Compounds of the invention may also antagonize the stimulation of gastric acid secretion produced by a standard dose of 1 nmole/kg pentagastrin. A compound also attenuates the amount of gastric acid

secreted in response to a 1 nmole/kg dose of pentagastrin (initial pentagastrin response 254 µmoles/l H^+, after the compound (cumulative dose of 1.1 µmole/kg) 128 µmoles/l H^+). With both compounds the antagonism is reversible with full recovery of the response to pentagastrin.

The compounds of the instant invention are also useful as antiulcer agents as discussed hereinbelow.

Aspirin-induced gastric damage is assessed in groups of 10 rats each.

All animals are fasted for 24 hours before and throughout the experiment. Drug or vehicle is given 10 minutes before an oral dose of 1 mL of a 45-mg/mL suspension of aspirin in 0.5% carboxymethylcellulose (CMC).

The animals are sacrificed five hours after aspirin administration and the stomachs removed and opened for examination.

Gastric damage was scored as follows:

Score	
1	Small hemorrhage
2	Large hemorrhage
3	Small ulcer
4	Large ulcer
5	Perforated ulcer

The mean ulcer score in the saline control group is 12.1 ± 6.85 (±SD). Treatment with ranitidine (15 mg/kg PO) inhibits ulcer formation by 74% giving an ulcer score of 3.2 ± 2.35 ($p < 0.001$ compared with controls). Treatment with a compound of the invention (10 mg/kg PO) results in an ulcer score of 6.3 ± 4.14 ($p < 0.05$ compared with controls), a 48% reduction in ulcer formation.

The specific dosages employed, however, may be varied depending upon the patient, the severity of the

condition being treated, and the activity of the compound employed. Determination of optimum dosages is within the skill of the art.

The compounds of the instant invention are also useful as anxiolytic agents as described and discussed below.

Anxiolytic activity is assessed in the light/dark exploration test in the mouse (B. J. Jones, et al, Br. J. Pharmacol. 93:985-993, 1988).

The number of mice is 5 and the pretreatment time is 40 minutes. The compound is given p.o. in 0.1, 1, and 10 mg/kg doses.

The apparatus is an open-topped box, 45 cm long, 27 cm wide, and 27 cm high, divided into a small (2/5) area and a large (3/5) area by a partition that extends 20 cm above the walls. There is a 7.5 x 7.5 cm opening in the partition at floor level. The small compartment is painted black and the large compartment white. The floor of each compartment is marked into 9 cm squares. The white compartment is illuminated by a 100-watt tungsten bulb 17 cm above the box and the black compartment by a similarly placed 60-watt red bulb. The laboratory is illuminated with red light.

All tests are performed between 13 hundred hours, 0 minutes and 18 hundred hours, 0 minutes. Each mouse is tested by placing it in the center of the white area and allowing it to explore the novel environment for five minutes. Its behavior is recorded on videotape and the behavioral analysis is performed subsequently from the recording. Five parameters are measured: the latency to entry into the dark compartment, the time spent in each area, the number of transitions between compartments, the number of lines

crossed in each compartment, and the number of rears in each compartment.

In this test an increase in the time spent in the light is a sensitive measure of, that is directly related to, the anxiolytic effects of several standard anxiolytic drugs. Drugs are dissolved in water or saline and administered either subcutaneously, intraperitoneally, or by mouth (PO) via a stomach needle.

Compounds are active by the subcutaneous route. Control animals show 3% crossings into the dark area over five-minute measurement periods. Mice treated with 1 mg/kg (SC) of a compound show 85 crossings into the light area and only 24 crossings into the dark area, a significant ($p < 0.01$) difference from the control anxious mice. Diazepam (0.25 mg/kg IP) has an effect identical to a compound in the same experiment.

The compounds of the instant invention are useful as antipsychotic agents. Compounds are tested for their ability to reduce the effects of intra-accumbens amphetamine in the rat as described hereinafter.

Male Sprague Dawley (CD) Bradford strain rats are used. The rats are housed in groups of five at a temperature of 21 ± 2°C on a 12 hour light-dark cycle of lights-on between 07 hours 00 minutes and 20 hours 00 minutes. Rats are fed CRM diet (Labsure) and allowed water ad libitum.

Rats are anesthetized with chloral hydrate (400 mg/kg SC) and placed in a Kopf stereotaxic frame. Chronically indwelling guide cannulae (constructed of stainless steel tubing 0.65 mm diameter held bilaterally in Perspex holders) are implanted using standard stereotaxic techniques to terminate 3.5 mm above the center of the nucleus accumbens (Ant. 9.4, Vert. 0.0, Lat. 1.6) or 5.0 mm above the central

nucleus of the amygdala (Ant. 5.8, Vert. −1.8, Lat. ±4.5) (atlas of De Groot, 1959). The guides are kept patent during a 14-day recovery period using stainless steel stylets, 0.3 mm diameter, which extend 0.5 mm beyond the guide tips.

Rats are manually restrained and the stylets removed. Intracerebral injection cannulae, 0.3 mm diameter, are inserted and drugs delivered in a volume of 0.5 µl over 5 seconds (a further 55 seconds was allowed for deposition) from Hamilton syringes attached via polythene tubing to the injection units. Animals are used on a single occasion only.

Behavioral experiments are conducted between 07 hours 30 minutes and 21 hours 30 minutes in a quiet room maintained at 22 ± 2°C. Rats are taken from the holding room and allowed one hour to adapt to the new environment. Locomotor activity is assessed in individual screened Perspex cages (25 x 15 x 15 cm (high) (banked in groups of 30) each fitted with one photocell unit along the longer axis 3.5 cm from the side; this position has been found to minimize spurious activity counts due to, for example, preening and head movements when the animal is stationary. Interruptions of the light beam are recorded every 5 minutes. At this time animals are also observed for the presence of any nonspecific change in locomotor activity, e.g., sedation, prostration, stereotyped movements, that could interfere with the recording of locomotor activity.

The abilities of compounds of the invention to inhibit the hyperactivity caused by the injection of amphetamine into the nucleus accumbens of the rat is measured.

An increase in locomotor activity follows the bilateral injection of amphetamine (20 µg) into the

nucleus accumbens; peak hyperactivity (50 to 60 counts 5 minutes^{-1}) occur 20 to 40 minutes after injection.

Intraperitoneal injection of the rats with a compound at 20 mg/kg or 30 mg/kg or at 10 mg/kg reduces the hyperactivity caused by the intra-accumbens injection of amphetamine. This test is known to be predictive of antipsychotic activity (Costall, Domeney & Naylor & Tyers, Brit J Pharmac 92:881-894).

EXAMPLE 1

N-[(1,1-dimethylethoxy)carbonyl]-α-methyl-DL-tryptophyl-L-methionyl-L-α-aspartyl-L-Phenylalaninamide

Boc-D-MeTrp-Met-Asp-Phe-NH_2 (1)

A solution of Asp-Phe-NH_2 (0.6 g, 2.02 mmol) in DMF (10 mL) was treated with diisopropylethylamine (261 mg, 2.02 mmol) followed by Fmoc-methionine pentafluorophenyl ester (Fmoc-Met-OPFP; 1.10 g, 2.05 mmol) and the mixture stirred overnight. Removal of the DMF was followed by trituration of the residue with diethyl ether:ethyl acetate (5:1) and the resulting solid filtered. This crude solid, Fmoc-Met-Asp-Phe-NH_2, could be used without further purification (1.22 g, 96%). Repeated to give 1.29 g of product. The solid prepared above (2.00 g, 3.16 mmol) was dissolved in DMF containing piperidine (20% to 50% v/v, 16 mL) and stirred for 20 minutes. Removal of the DMF and piperidine was followed by trituration of the residue with ether, yielding a white solid, Met-Asp-Phe-NH_2, in quantitative yield. This too could be used further without purification.

The free tripeptide (205 mg, 0.5 mmol) was suspended in DMF (5 mL) and diisopropylethylamine

(129 mg, 1.0 mmol) added. Boc-DL-MeTrp-OPFP (242 mg, 0.5 mmol) was added and the mixture stirred until clear (approx. 2 days). Removal of volatiles left a light tan residue which was triturated with diethyl ether: ethyl acetate (1:1) giving a near white solid. Chromatography of this solid (silica gel, 5% MeOH in dichloromethane with 1% AcOH) yielded a near white solid, 279 mg, 78% yield.

NMR (DMSO-d_6 δ 1.21 and 1.28 (3H, 2s), 1.42 and 1.43 (9H, 2s), 1.99 and 2.01 (3H, 2s), 2.24-2.54 (8H, m), 2.81 (1H, dd), 3.11 (2H, dm), 4.18-4.4 (3H, m), 4.5 (1H, m), 6.78 (1H, br.s), 6.86-7.34 (11H, m), 7.47 (1H, d), 7.52 and 7.41 (1H, 2br.s), 7.7 (1H, br.m), 8.06 (1H, br.t), 8.24 (1H, br.d).

FAB-MS (+ve ion) 749 (M+K), 733 (M+Na), 711 (M+H)
(-ve ion) 709 (M-H)

Microanalysis; Calc. for $C_{35}H_{46}N_6O_8S \cdot 0.75\ H_2O$:
C, 58.03; H, 6.54; N, 11.60; S, 4.42.
Found: C, 58.13; H, 6.48; N, 11.35; S, 4.40.

Similarly prepared was N-[[(1,1-dimethylethoxy)-carbonyl]-α-methyl]-L-tryptophyl]-L-methionyl-L-α-aspartyl-L-phenylalaninamide

Boc-L-α-MeTrp-Met-Asp-Phe-NH_2, (2), and

N-[(1-1-dimethylethoxy)carbonyl]-α-methyl-D-tryptophyl-L-methionyl-L-α-aspartyl-L-phenylalaninamide

Boc-D-α-MeTrp-Met-Asp-Phe-NH_2, (3).

NMR (2; DMSO-d_6) δ 1.25 (3H, s), 1.44 (9H, s), 1.83 (2H, m), 2.01 (3H, s), 2.38 (2H, m), 2.65 (1H, m), 2.82 (1H, m), 4.20 (1H, m), 4.33 (1H, m), 4.50 (1H,

m), 6.92-7.47 (12H, m), 7.82 (2H, m), 8.11 (1H, br.d), 10.91 (1H, s).

NMR (3; DMSO-d_6) δ 1.29 (3H, s), 1.44 (9H, s), 1.92 (2H, m), 2.02 (3H, s), 2.42 (2H, t), 2.60 (1H, td), 2.84 (1H, dd), 3.13 (2H, d), 3.31 (2H+water), 4.19 (1H, m), 4.36 (1H, m), 4.48 (1H, m), 6.94-7.24 (12H, m), 7.32 (1H, d), 7.47 (1H, d), 7.84 (1H, br.d), 8.14 (1H, br.d), 8.22 (1H, br.s), 10.92 (1H, s).

EXAMPLE 2

Also prepared by this method were α-methyl-L-tryptophyl-L-methionyl-L-α-aspartyl-L-phenylalaninamide

L-α-MeTrp-Met-Asp-Phe-NH_2, (4),

α-methyl-D-tryptophyl-L-methionyl-L-α-aspartyl-L-phenylalaninamide

D-α-MeTrp-Met-Asp-Phe-NH_2, (5) and

α-methyl-DL-tryptophyl-L-methionyl-L-α-aspartyl-L-phenylalaninamide

DL-α-MeTrp-Met-Asp-Phe-NH_2, (6).

In these latter three instances the final residue used was the appropriate Fmoc-α-MeTrp activated ester leading to the protected tetrapeptide which was treated with piperidine in DMF to remove the protecting group. This furnished the completely deprotected tetrapeptides (4-6). Purification was by RP-HPLC (MeCN-H_2O/0.1% TFA) yielding the trifluoroacetate salt of each peptide.

NMR (4; D_2O) δ 1.73 (3H, s), 1.85 (2H, q), 2.04 (3H, s), 2.34 (2H, t), 2.66 (2H, qd), 3.07 (2H, qd), 3.41 (2H, s), 4.34 (1H, t), 4.54 (2H, m), 7.14 (1H, t), 7.21-7.37 (9H, m), 7.48-7.56 (2H, dd).

NMR (5; D_2O) δ 1.54 (3H, s), 1.78 (2H, m), 1.97 (3H, s), 2.54 (2H, m), 2.93-3.28 (6H, m), 4.13 (1H, t), 4.42 (1H, t), 4.55 (1H, m), 7.13-7.36 (8H, m), 7.51 (1H, d), 8.18 (1H, d).

NMR (6; D_2O) δ 1.73 (3H, s), 1.85 (2H, m), 1.94 and 2.03 (3H, 2s), 2.34 (1H, t), 2.74 (2H, m), 3.06 (2H, qd), 3.40 (2H, abq), 4.01 and 4.34 (1H, 2t), 4.60 (2H, m), 7.11-7.48 (10H, m), 7.53 (2H, t).

The following compounds were made by the processes described above for Method A.

Glycyl-α-methyl-D-tryptophyl-L-methionyl-L-α-aspartyl-L-phenylalaninamide (Gly-DMeTrp-Met-Asp-Phe-NH_2),

Glycyl-α-methyl-DL-tryptophyl-L-methionyl-L-α-aspartyl-L-phenylalaninamide (Gly-DL-MeTrp-Met-Asp-Phe-NH_2),

Glycyl-α-methyl-L-tryptophyl-L-methionyl-L-α-aspartyl-L-phenylalaninamide (Gly-L-MeTrp-Met-Asp-Phe-NH_2),

Trp-Met-Asp-DL-MePhe-$NH_2 \cdot CF_3CO_2H$, and

Trp-Met-DL-MeAsp-Phe-$NH_2 \cdot CF_3CO_2H$.

They can also be made by the following example.

EXAMPLE 3

L-tryptophyl-α-methyl-DL-methionyl-L-α-aspartyl-L-phenylalaninamide

L-Trp-DL-α-MeMet-Asp-Phe-NH_2 (7)

Using a Nova Biochem 4170 automated peptide synthesizer and Bioplus® software the peptide was constructed from the C-terminus using Fmoc amino acid pentafluorophenyl or DHBt esters and HOBt catalysis. Each residue was present in a fivefold excess to ensure rapid and complete acylation. On a 0.095 mmol scale, the peptide (4) was isolated following TFA cleavage (94% TFA, 5% anisole, 1% ethanedithiol) from the resin (2h, room temperature) in 46% yield (32 mg isolated). HPLC (C18, 20-80% MeCN-H_2O + 0.1% TFA) showed greater than 90% purity (254 nm detection) as a 1:1 mixture of diastereomers.

NMR (D_2O) δ 1.03 (3H, s), 1.69 (2H, m), 1.91 (2H, m), 1.95 (3H, s), 2.59 (2H, m), 3.09 (2H, m), 3.38 (@H, m), 4.31 (1H, m), 4.51 (2H, m), 7.17-7.37 (10H, m), 7.52 (1H, d), 7.68 (1H, d), 10.94 (1H, s). When produced by solution-phase methods the product was identical.

Similarly prepared was L-α-MeTrp-Met-Asp-Phe-NH_2, (4), identical in every respect to that prepared above.

EXAMPLE 4

Trp-Met-Asp-DL-MePhe-$NH_2 \cdot CF_3CO_2H$ (8)

Fmoc-Asp(OBu^t)-DL-MePhe-NH_2

A mixture of MePhe-NH_2 (90 mg, 0.5 mmol) and Fmoc-Asp(OBu^t)OPfp (290 mg, 0.5 mmol) were dissolved in the minimum quantity of ethyl acetate and the solvent

immediately distilled away. The resulting viscous oil was heated in a boiling water bath for 2 hours, then cooled. The resulting resin was triturated with diethyl ether from which the product precipitated. Yield 159 mg (56%). A further crop of product could be obtained by chromatography of the ethereal washings.

Fmoc-Met-Asp(OBu^t)-DL-MePhe-NH$_2$

The preceding dipeptide (900 mg, 1.58 mmol) was N-deprotected using 20% piperidine in DMF for 20 minutes then the solvent removed completely. The residue was repeatedly triturated with n-hexane leaving the free amino dipeptide (293 mg, 0.84 mmol). To this was added Fmoc-Met-OPfp (450 mg, 0.84 mmol) followed by the minimum quantity of ethyl acetate to give a clear solution. The solvent was immediately removed and the resulting viscous oil heated in a water bath at 65°C for 45 minutes. Trituration of the resulting resin with diethyl ether yielded the protected tripeptide (418 mg, 78%).

Fmoc-Trp-Met-Asp(OBu^t)-DL-MePhe-NH$_2$

The preceding tripeptide (300 mg, 0.43 mmol) was N-deprotected using 20% piperidine in DMF for 20 minutes. Removal of the solvent followed by trituration of the residue with n-hexane left the free tripeptide (198 mg, 0.41 mmol). To this was added Fmoc-Trp-OPfp (243 mg, 0.41 mmol) and sufficient ethyl acetate to give a clear solution. The solvent was immediately removed and the resulting mixture heated at 65°C for 15 minutes. Trituration of the residue with diethyl ether gave a white solid, 283 mg, 78%.

Trp-Met-Asp-<u>DL</u>-MePhe-$NH_2 \cdot CF_3CO_2H$ (8)

The preceding tetrapeptide was N-deprotected using 20% piperidine in DMF for 20 minutes then the solvent removed and the residue triturated with n-hexane, leaving the free amino tetrapeptide. This was treated with thioanisole and ethanedithiol (100-250 µL/mmol and 50-100 µL/mmol) followed by 95% aqueous TFA (3-5 mL/mmol) for 5 minutes. All volatiles were removed and the residue triturated with diethyl ether, giving a white solid, purified by preparative HPLC.

Trp-Met-<u>DL</u>-MeAsp-Phe-$NH_2 \cdot CF_3CO_2H$ (9)

Fmoc-<u>DL</u>-MeAsp(OBu^t)-Phe-NH_2

Fmoc-<u>DL</u>-MeAsp(OBu^t)OPfp (1.46 g, 2.47 mmol) and H-Phe-NH_2 (0.41 g, 2.5 mmol) were dissolved in the minimum quantity of ethyl acetate to give a clear solution, then the solvent was immediately removed. The mixture was then heated at 100°C for 1 hour and cooled. Trituration of the residue with diethyl ether yielded the protected dipeptide, 993 mg, 71%.

Fmoc-Met-<u>DL</u>-MeAsp(OBu^t)-Phe-NH_2

The preceding dipeptide (571 mg, 1.0 mmol) was N-deprotected as previously described. Following trituration with n-hexane, the residue was mixed with Fmoc-Met-OPfp (350 mg, 0.65 mmol) and sufficient ethyl acetate to dissolve. Immediate removal of the solvent was followed by heating the residue at 65°C for 4 hours. Trituration of the residue with diethyl ether yielded the protected tripeptide 210 mg, 46%. A further crop could be isolated from the ethereal washings by chromatography.

Fmoc-Trp-Met-DL-MeAsp(OBu^t)-Ph-NH_2

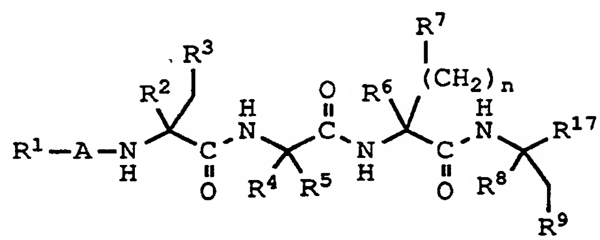
The above tripeptide (400 mg, 0.57 mmol) was N-deprotected as previously described. Following trituration of the residue with n-hexane, 258 mg of product were isolated. 200 mg (0.42 mmol) of this and Fmoc-Trp-OPfp (246 mg, 0.42 mmol) was added with sufficient ethyl acetate to dissolve. The solvent was immediately removed and the residue heated at 65°C for 20 minutes. Trituration of the resulting mixture with diethyl ether yielded the protected tetrapeptide, 283 mg, 77%.

Trp-Met-DL-MeAsp-Phe-$NH_2 \cdot CF_3CO_2H$ (9)

The preceding tetrapeptide was treated as the tetrapeptide, yielding (8) and gave after final trituration with ether.

CLAIMS

1. A compound of formula



I

or a pharmaceutically acceptable salt thereof wherein

R^1 is hydrogen, BOC, 1-Adoc, 2-Adoc, Z, FMOC, $R^{12}R^{13}R^{14}Si(CH_2)_mOCO-$ wherein R^{12}, R^{13}, and R^{14} are each independently lower alkyl of from 1 to 3 carbon atoms and m is an integer of from 0 to 3, $R^{15}CO-$ or $R^{15}NHCO-$ wherein R^{15} is a straight or branched alkyl of from 1 to 6 carbons or 1- or 2-adamantyl;

A is a bond, Gly, ß-ALA, GABA, DAVA, Aib, $NH(CH_2)_p$ wherein p is an integer of from 1 to 6, NHCO, Met-Gly, Tyr-Met-Gly, or Asp-Tyr-Met-Gly, Asp-Tyr(OSO_3H)-Met-Gly;

R^2, R^4, R^6, and R^8 are each independently hydrogen with the proviso that not all are simultaneously hydrogen, lower alkyl, $-CH=CH_2$, $C\equiv CH$, $-CH_2-CH=CH_2$, $-CH_2C\equiv CH$, $-CH_2Ar$, $-CH_2OR$, $-CH_2OAr$, $-(CH_2)_qCO_2R$, $-(CH_2)_qN(R)_2$ wherein q is an integer of from 0 to 3, R is hydrogen or lower alkyl, Ar is a mono- or polycyclic unsubstituted or substituted carbo- or heteroaromatic or hydroaromatic moiety;

R^3 is 2- or 3-indolyl, 1- or 2-naphthyl, 2- or 3-benzothienyl, 2- or 3-benzofuranyl, 2- or 3-indazoyl or phenyl with from 0 to 3 substituents each independently selected from hydrogen, fluorine, chlorine, bromine, iodine, methyl, methoxy, trifluoromethyl, nitro, hydroxy, NH_2, and OCF_3;

R^5 is straight or branched lower alkyl of from 1 to 6 carbon atoms, $(CH_2)_rS(CH_2)_sCH_3$ wherein r is an integer of from 1 to 6 and s is an integer of from 0 to 6;

n is an integer of from 1 to 3;

R^7 is -OH,
-COOH,
tetrazole,
triazole,
$-COOR^{16}$,
$-CONR^{10}R^{11}$,
wherein R^{16} is lower alkyl, and R^{10} and R^{11} are as described below;

R^9 is selected from R^3 above;

R^{17} is $-\overset{O}{\overset{\|}{C}}-NR^{10}R^{11}$, CH_2OR^{18} or H wherein

R^{10} and R^{11} are each independently hydrogen, lower alkyl, alkoxycarbonyl, carboxyalkyl, or R^{10} and R^{11} together form a ring of from 3 to 10 atoms with the nitrogen to which they are attached which ring contains atoms selected from carbon, nitrogen, oxygen, and sulfur;

R^{18} is R^1 or CH_2CO_2H or $CH_2CONR^{10}R^{11}$ or $CONR^{10}R^{11}$;

R^2 and R^3, R^4 and R^5, R^6 and R^7, R^8 and R^9 or R^9 and R^{10} may together form a ring of from 3 to 8 atoms which atoms are selected from carbon, nitrogen, oxygen, and sulfur.

2. A compound according to Claim 1 wherein R^1 is Fmoc, Z, Boc, or $Me_3Si(CH_2)_2OCO_2H$; A is a bond, Gly, ß-ALA, Asp-Tyr-Met-Gly; R^2, R^4, R^6, and R^8 are each independently alkyl of from 1 to 3 carbon atoms, carboxyalkyl of 1 or 2 carbon atoms, or CH_2OH; R^3 is 3-indazolyl, 3-benzothienyl, 2-benzofuranyl, 3-benzofuranyl, or 2-bromo-3-benzofuranyl; R^5 is isobutyl, sec-butylethyl, n-propyl, or i-propyl; R^7 is $CONH_2$, $CONMe_2$, or OH; R^9 is selected from R^3; R^{17} is $-\overset{\displaystyle O}{\overset{\|}{C}}-NR^{10}R^{11}$, wherein R^{10} and R^{11} are each independently alkyl of from 1 to 3 carbon atoms or together R^{10} and R^{11} form a ring of from 4 to 8 atoms including the nitrogen to which they are attached.

3. A compound according to Claim 1 wherein R^1 is hydrogen or BOC; A is a bond or ß-ALA; R^2, R^4, R^6, and R^8 are each independently CH_3 or CH_2CO_2H; R^3 is 3-indolyl, 1-naphthyl, or 2-naphthyl; R^5 is n-butyl, n-pentyl, $(CH_2)_2SCH_3$ or $(CH_2)_2SCH_2CH_3$; R^7 is OH, tetrazole, triazole, or CO_2H; R^9 is phenyl, 1-naphthyl, or 2-naphthyl; R^{17} is $-\overset{\displaystyle O}{\overset{\|}{C}}-NR^{10}R^{11}$, wherein R^{10} and R^{11} are each independently hydrogen, CH_3

or together R^{10} and R^{11} form a ring of 5 or 6 atoms including the nitrogen to which they are attached.

4. A compound named α-methyl-DL-tryptophyl-L-methionyl-L-α-aspartyl-L-phenylalaninamide.

5. A compound named α-methyl-D-tryptophyl-L-methionyl-L-α-aspartyl-L-phenylalaninamide.

6. A compound named α-methyl-L-tryptophyl-L-methionyl-L-α-aspartyl-L-phenylalaninamide.

7. A compound named N-[[(1,1-dimethylethoxy)-carbonyl]-α-methyl]-L-tryptophyl]-L-methionyl-L-α-aspartyl-L-phenylalaninamide.

8. A compound named N-[(1,1-dimethylethoxy)-carbonyl]-α-methyl-DL-tryptophyl-L-methionyl-L-α-aspartyl-L-phenylalaninamide.

9. A compound named L-tryptophyl-2-methyl-DL-methionyl-L-α-aspartyl-L-phenylalaninamide.

10. A compound named N-[(1,1-dimethylethoxy)-carbonyl]-α-methyl-D-tryptophyl-L-methionyl-L-α-aspartyl-L-phenylalaninamide.

11. A compound named glycyl-α-methyl-D-tryptophyl-L-methionyl-L-α-aspartyl-L-phenylalaninamide.

12. A compound named glycyl-α-methyl-DL-tryptophyl-L-methionyl-L-α-aspartyl-L-phenylalaninamide.

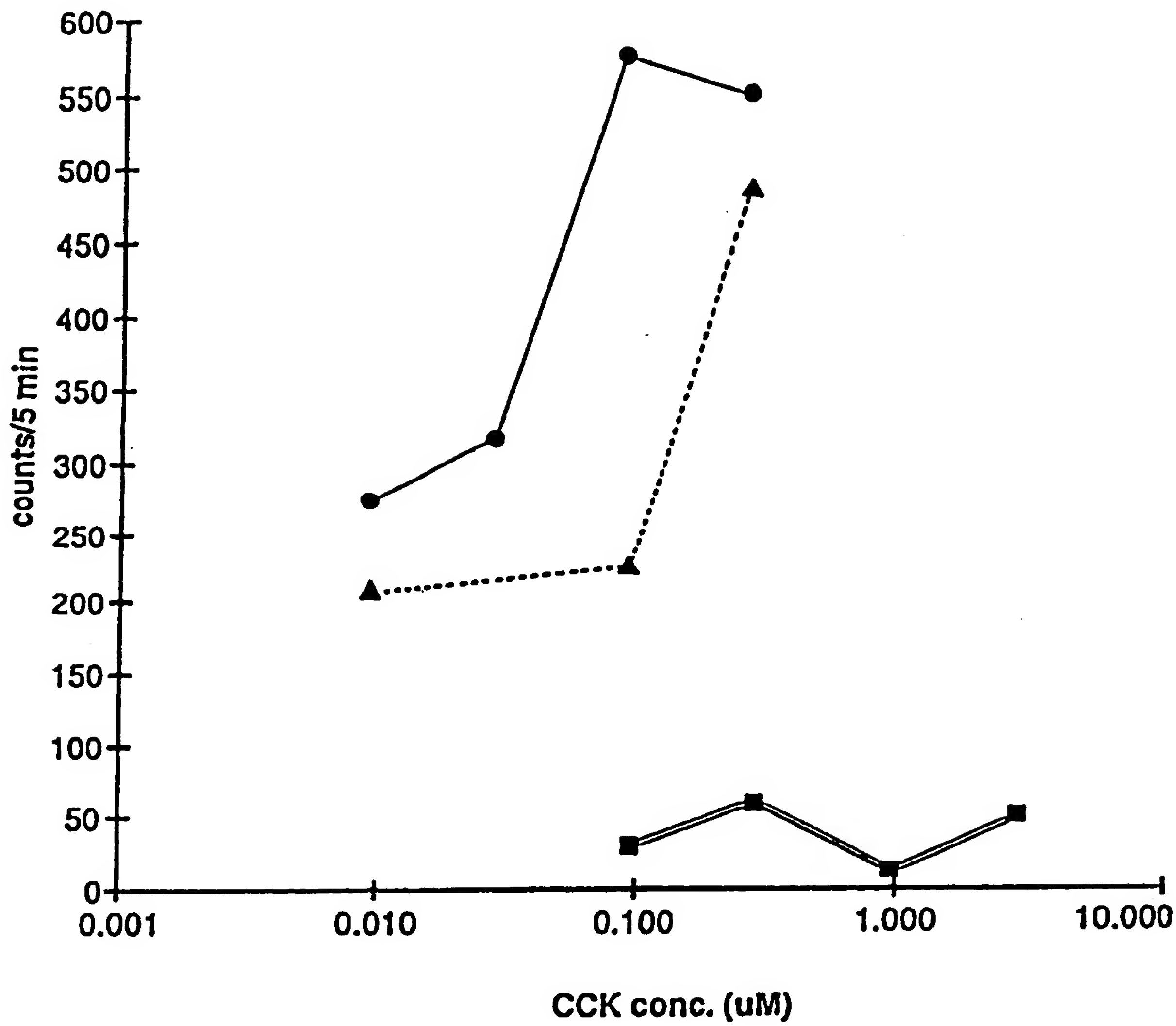
13. A compound named glycyl-α-methyl-L-tryptophyl-L-methionyl-L-α-aspartyl-L-phenylalaninamide.

14. A pharmaceutical composition comprising an amount of a compound according to Claim 1, effective to suppress the appetite in a mammal, and a pharmaceutically acceptable carrier.

15. A method of suppressing appetite in a mammal, comprising administering an effective appetite suppressing amount of a compound according to Claim 1.

16. A compound selected from:
Fmoc-MePhe-NH_2,
Fmoc-Asp(OBu^t)-MePhe-NH_2,
Fmoc-Met-Asp(OBu^t)-MePhe-NH_2,
Fmoc-Trp-Met-Asp(OBu^t)-MePhe-NH_2,
Asp(OBu^t)-MePhe-NH_2,
Asp-MePhe-NH_2,
Fmoc-Met-Asp-MePhe-NH_2,
Met-Asp(OBu^t)-MePhe-NH_2,
Met-Asp-MePhe-NH_2,
Trp-Met-Asp(OBu^t)-MePhe-NH_2,
Fmoc-Trp-Met-Asp-MePhe-NH_2,
Fmoc-MeAsp(OBu^t)-Phe-NH_2,
Fmoc-MeAsp-Phe-NH_2,
Fmoc-Met-MeAsp(OBu^t)-Phe-NH_2,
Fmoc-Trp-Met-MeAsp(OBu^t)-Phe-NH_2,
MeAsp(OBu^t)-Phe-NH_2,
MeAsp-Phe-NH_2,
Met-MeAsp(OBu^t)-Phe-NH_2,
Met-MeAsp-Phe-NH_2,
Fmoc-Met-MeAsp-Phe-NH_2,
Fmoc-Trp-Met-MeAsp-Phe-NH_2,

MeMet-Asp(OBu^t)-Phe-NH_2,
Fmoc-MeMet-Asp(OBu^t)-Phe-NH_2,
Fmoc-MeMet-Asp-Phe-NH_2
MeMet-Asp-Phe-NH_2,
Fmoc-Trp-MeMet-Asp(OBu^t)-Phe-NH_2,
Fmoc-Trp-MeMet-Asp-Phe-NH_2,
Fmoc-MeTrp-Met-Asp(OBu^t)-Phe-NH_2,
Fmoc-MeTrp-Met-Asp-Phe-NH_2,
Fmoc-Gly-Trp-Met-Asp-MePhe-NH_2,
Fmoc-Gly-Trp-Met-Asp(OBu^t)MePhe-NH_2,
Gly-Trp-Met-Asp(OBu^t)MePhe-NH_2,
Fmoc-Gly-Trp-Met-MeAsp-Phe-NH_2,
Fmoc-Gly-Trp-Met-MeAsp(OBu^t)-Phe-NH_2,
Gly-Trp-Met-MeAsp(OBu^t)-Phe-NH_2,
Fmoc-Gly-Trp-MeMet-Asp-Phe-NH_2,
Fmoc-Gly-Trp-MeMet-Asp(OBu^t)-Phe-NH_2,
Gly-Trp-MeMet-Asp(OBu^t)-Phe-NH_2,
Fmoc-Gly-MeTrp-Met-Asp-Phe-NH_2,
Fmoc-Gly-MeTrp-Met-Asp(OBu^t)-Phe-NH_2,
Gly-MeTrp-Met-Asp(OBu^t)-Phe-NH_2,
Fmoc-MeMet-OH,
Fmoc-MeMet-OPFP,
MeAsp(OBu^t)OH,
Fmoc-MeAsp(OBu^t)OH, and
Fmoc-MeAsp(OBu^t)OPFP.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CCK conc. (uM)

FIG. I

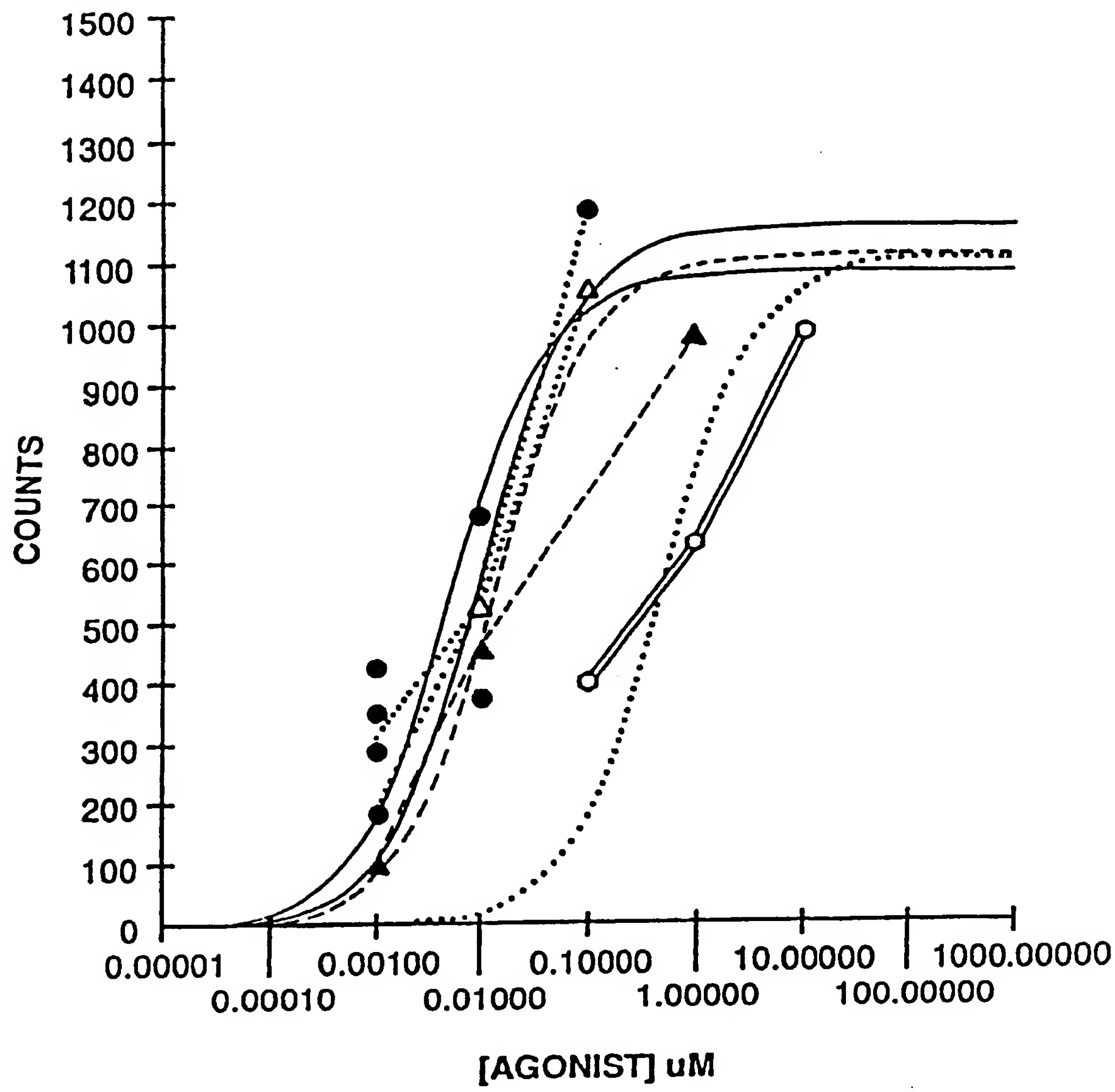
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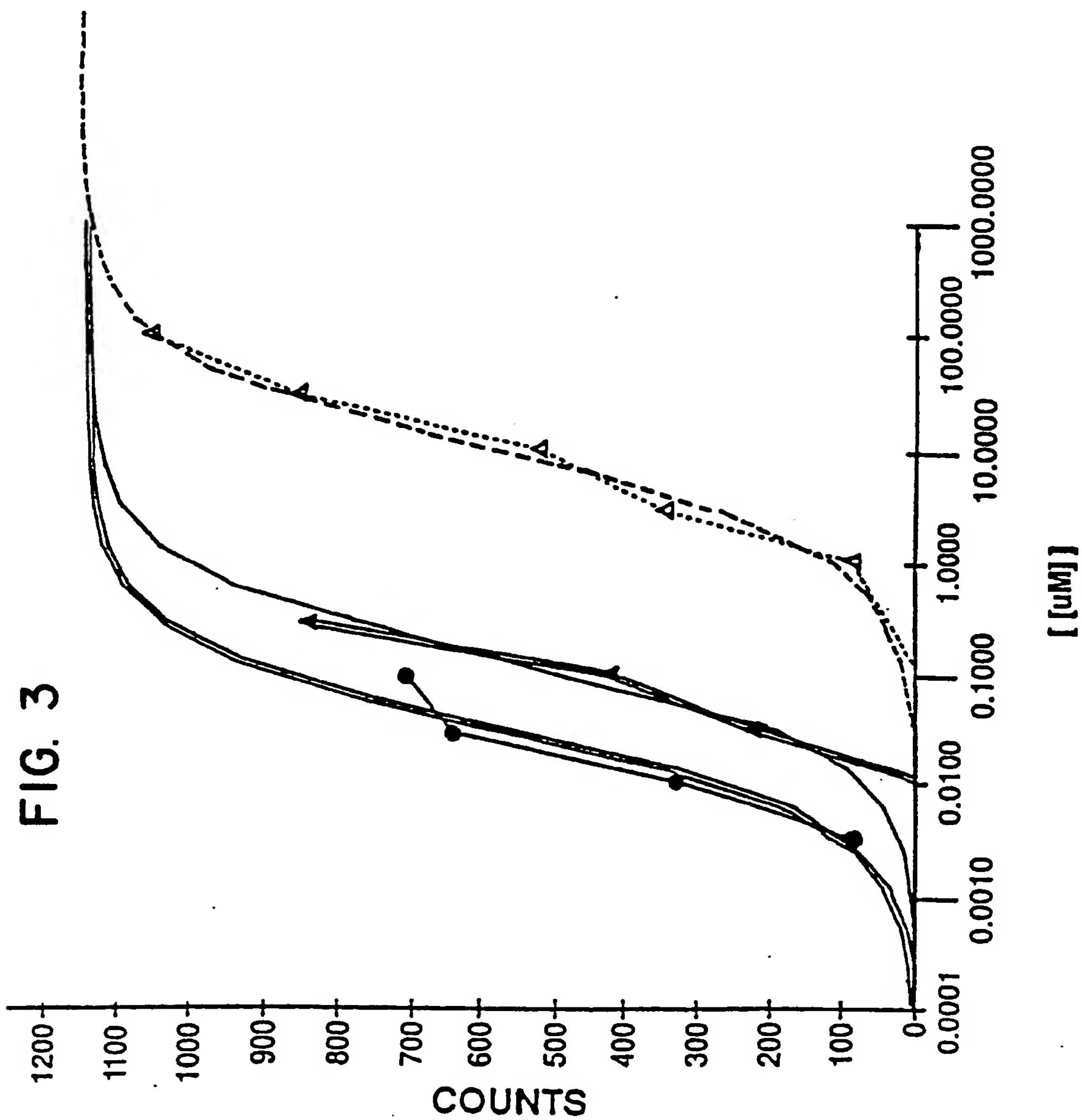
FIG. 2

FIG. 3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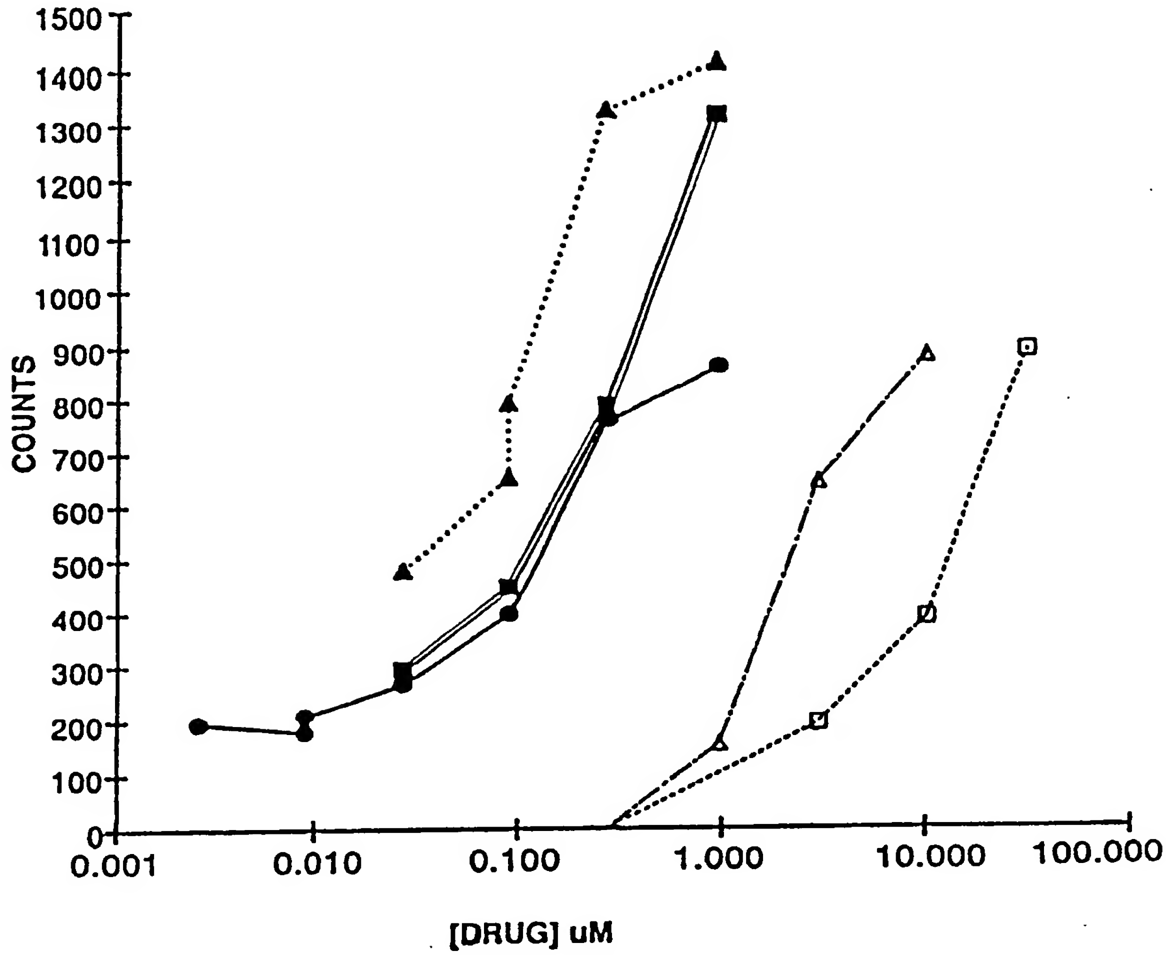
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[DRUG] uM

FIG. 4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/03015

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 37/00; C07K 5/0?
US CL :514/16, 17, 18; 530/328, 329, 330
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/16, 17, 18; 530/328, 329, 330

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS ONLINE, APS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	EP, A, 0,226,217 (Rosamond) 24 June 1987. See pages 25, 26, 38 and 47.	1,14,15 1-16
Y	WO, A, 90/12810 (Rivier et al.) 01 November 1990. See pages 1-2.	1-16
Y,P	US, A, 5,013,722 (Danho et al.) 07 May 1991. See column 2, lines 8-16.	1-16
Y	Chemical and Pharmaceutical Bulletin of Japan, Volume 36(9), issued 1988, Fujii et al., "Studies on Peptides. CLIX.1.2 Preparation of a Protected 33-residue peptide for the synthesis of human cholecystokinin (hCCK-33)" pages 3271-3280. See entire document.	1-16

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be part of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier document published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search 23 JULY 1992	Date of mailing of the international search report 29 JUL 1992
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